Plant-Soil Interactions and Implications for Restoration of Coastal Sand Dunes in Point Reyes National Seashore

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ABSTRACT

The invasion of exotic species is one of the most predominant threats to biodiversity worldwide. *Carpobrotus edulis* and *Ammophila arenaria* are two common invasives at Point Reyes National Seashore, where restoration projects are done to protect the sensitive dune habitat and rare species. Methods of restoration I studied are manual pulling of *C. edulis* and a mechanical flipping of sand to bury *A. arenaria*. I examined the effects of plant-soil feedbacks after types of restoration of *C. edulis* and *A. arenaria*, and how the changes may affect the re-growth of two rare plants, *Layia carnosa* and *Monardella undulata*. I ran three studies: 1) Soil nutrient analyses under native and *C. edulis* dune soil; 2) A greenhouse study of whole field sand to examine the growth of the two native species in three types of restored soil and in native soil; and 3) An inoculum greenhouse study to specifically test for microbial interactions. Significantly more seeds germinated in native soil than the flipped treatment, with minimal germination in the *C. edulis* treatments. The greenhouse studies show that removing *C. edulis* duff can help the growth of native plants, while plants grew significantly less in the flipped treatment than in the native soil. I found similar effects in the microbe study, which shows microbes ought to be considered as plant-soil feedback when performing and monitoring restoration projects. The results of this study will be used by Point Reyes National Seashore to guide restoration projects to benefit native dune plants.

KEYWORDS

Carpobrotus edulis, *Ammophila arenaria*, habitat restoration, invasive species, soil parameters, alien plants, seedling establishment

INTRODUCTION

The invasion and establishment of introduced species is one of the biggest threats to biodiversity worldwide. Invasion can lead to homogenization of plant communities by a single species, as invasives compete directly for space and may alter ecosystem processes to facilitate their own growth (D'Antonio and Vitousek 1992). Impacts of invasions on plant diversity arise through the alteration of ecosystem variables and processes such as nutrient cycling and disturbance regimes (D'Antonio and Vitousek 1992, Levine 2002, Ehrenfeld 2003). Studying exotic species can provide valuable knowledge of how ecosystem processes such as nutrient cycling, soil development, and hydrology may be controlled by plants (Vitousek 1990). The ecological impacts of invasive plant species can be caused by various pathways of competition.

Plant-soil feedbacks from invasive exotic species must be taken into account in restoration projects because invasive species may change soil nutrients or soil microbial communities (Bever 1994, Ehrenfeld 2003). Changes in soil nutrient cycles may be a result of changing vegetation that causes a shift in the microbe community as a result of different type and quantity of inputs to the soil (Ehrenfeld 2003). The composition of soil microbial communities has large effects on the interactions between plants and on diversity. Modification of plant interactions may alter plant-soil feedbacks through changes in beneficial or detrimental microbes (Bever et al. 2010). Diagnosis of the pathways of feedback modifications are essential for restoration, as residual below-ground changes could impede the recolonization of native plants (Ehrenfeld 2003, Conser and Connor 2008). The management of coastal dunes often involves restoration, as they are prone to invasion.

Coastal dune habitats in California are extensively colonized by invasive species, providing specific challenges for restoration and management. *Carpobrotus edulis*—highway iceplant—is a succulent mat-forming plant from South Africa and is a prominent invasive species in California. It establishes a soil environment that enhances its own growth and negatively affects the growth of native plants (Conser and Connor 2009, de la Peña et al. 2010). Soils from areas with long-term *C. edulis* establishment have a lower pH and higher organic matter content compared to areas that are not invaded (de la Peña et al. 2010, Santoro et al. 2011). The soil conditions also negatively impact the growth of at least one rare native plant after removal in restoration (Conser and Connor 2009). In addition, pioneer habitats with poor soils are more sensitive to invasion, possibly as a result of the relatively higher amount of litter produced by *C. edulis*, which may be the cause of higher organic matter and soil nitrogen (Santoro et al. 2011). Therefore it is important to address the soil environment after restoration projects (Conser and Connor 2009). However, little is known of how the approach of restoration affects the succession of natives after *C. edulis* is removed.

The objective of this research is to examine how *C. edulis* impacts dune habitats and native species, and investigate what the best solutions are to recover or prevent further loss of sensitive native dune habitat at Point Reyes National Seashore. I ran soil nutrient analyses and two greenhouse studies to look at the effects of soil type on the growth of native plants, testing entirely field sand and a more specific microbe inoculum. In this study, I seek to answer the questions: 1) How does *C. edulis* change soil nutrients and affect plant communities? and 2) What type of restoration at Point Reyes National Seashore will enable the most effective reestablishment of the endangered or rare native plants *Layia carnosa* and *Monardella undulata*? The results of this study will be used by Point Reyes National Seashore to effectively manage *C. edulis* in the dunes and to guide restoration projects to be most beneficial to native dune plants.

METHODS

Site description and study subjects

I conducted this study on the dunes of the Great Beach at Point Reyes National Seashore (PRNS) in Marin County of Northern California (38°6'N, 122°57'W) (Fig. 1). Point Reyes has a Mediterranean climate with summer fog and mild winter months. The western dunes have strong winds, with an average annual maximum of 43 mph.

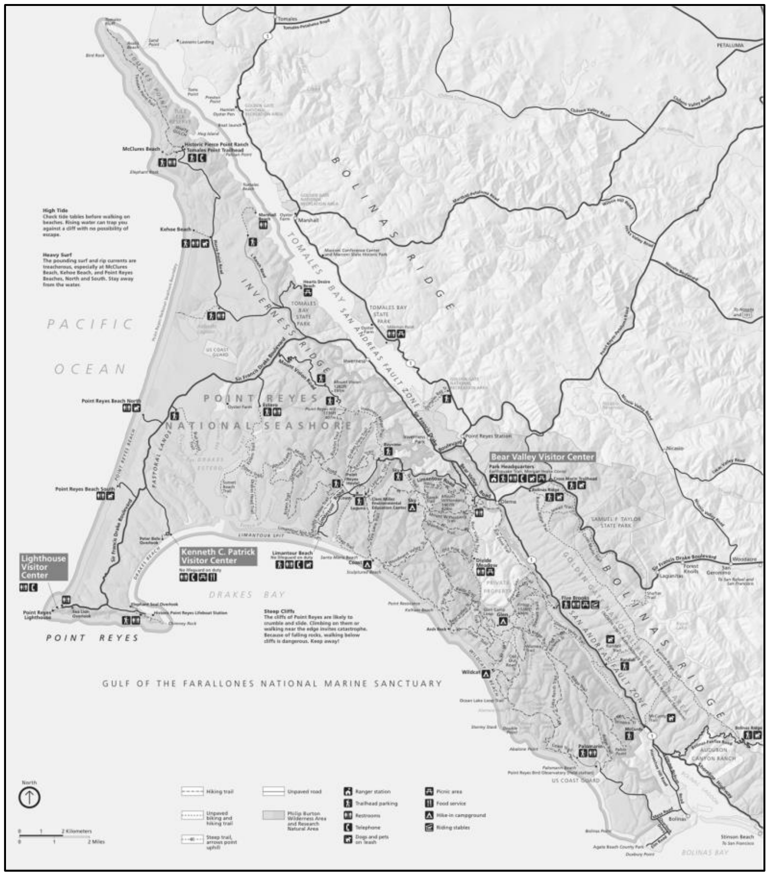

Fig. 1. Map of Point Reyes National Seashore in Marin County, California. Study site is located along the western beach between Abbott's Lagoon and North Beach.

Native plant communities in the dunes have been extensively invaded by exotic plants. *Carpobrotus edulis*, highway iceplant, and *A. arenaria* were first introduced in Point Reyes by ranchers in the 1940s to stabilize the dunes and prevent sand from moving into the grasslands. Of more than 1,400 acres of dunes, over 1,000 (70%) are established with *C. edulis* and *A. arenaria* (Minnick 2012). Both species exclude other plants from growing and prevent sand from moving in this naturally mobile system.

The main activities in the dune area are restoration projects and monitoring for the endangered plants and the wildlife such as the threatened Western snowy plover. Most restoration projects in the dunes involve pulling *C. edulis* or spraying with herbicide. The Abbott's Lagoon Restoration Project of Spring-Fall 2011 removed 80 acres of *A. arenaria*, European beachgrass, with machines to dig trenches and bury the plants under at least 9 feet of bare, flipped sand; as a result, this area was left highly mobile with no stabilization or plant cover.

Of the diverse, highly specialized plants that grow in coastal sand dunes, I studied two species, *Layia carnosa* and *Monardella undulata*. The California state and federally endangered *L. carnosa* is an annual distributed between Santa Barbara County and Humboldt County with variable, patchy density. It grows in semi-stable sand ridges and troughs among dune mat vegetation. It germinates in the fall and seedling density peaks in the winter before other species (Basor 2002). *Monardella undulata* is a California endemic distributed between Santa Barbara County and Sonoma County, and is classified as rare by the California Native Plant Society (www.rareplants.cnps.org). It is also an important nectar species for the endangered Myrtle's silverspot butterfly. No research has been done on growing characteristics.

Characterizing Soil Chemistry & Composition

To measure the effects of *C. edulis* on soil nutrients and chemistry along a gradient of invasion, I collected soil samples from native and invaded communities from the area between North Beach and Abbott's Lagoon at PRNS. I mapped *L. carnosa* occurrences as well as *C. edulis* cover adjacent to *L. carnosa* populations. This mapping allowed me to select random areas for the following soil experiments using ArcMap GIS and a random number chart. I located 11 areas with similar abiotic conditions (including distance from shore, exposure to wind, and elevation) where *C. edulis* grew directly adjacent to *L. carnosa* occurrences without other plants along the edge; patches were chosen with the same topographic position (slope, aspect, altitude) between species areas so there would be no reason to suspect the two plant communities had different abiotic conditions (Santoro et al. 2011).

To measure soil effects along a gradient of invasion of *C.* edulis, I sampled three soil types: (1) in the center of a large, established patch of *C. edulis*, (2) the edge of the same patch, where *C. edulis* is rooting but does not have a duff layer, and (3) the adjacent native dune where *L. carnosa* has been found. Within each of the soil types in a localized area I combined three sub-samples into one container to be mixed thoroughly. I collected each sub-sample by pounding a tube four cm in diameter into the ground and emptying it into a bag to be mixed with other sub-samples. I sent samples of about 475 ml to A&L Western Agricultural Lab in Modesto, California (http://www.al-labs-west.com/) to be analyzed for organic matter, Nitrate-Nitrogen, Phosphorus (Weak Bray and Sodium Bicarbonate-P), extractable cations (Potassium, Magnesium, Calcium, Sodium), Hydrogen, Sulfate-S, pH, and cation exchange capacity; many of these are commonly used to analyze effects of plant invasion on soil processes (Ehrenfeld 2003, Santoro et al. 2011). To test for differences in soil properties from *C. edulis*, I performed one-way ANOVAs on each outcome variable by soil type. For significant ANOVA results, I performed a post-hoc Tukey HSD test to determine which groups were significantly different from each other.

Restored soils and native plant growth

I used greenhouse experiments to evaluate what types of restoration provide soil conditions that allow for the most effective reestablishment of native plant species. To determine if *L. carnosa* and *M. undulata* reestablish in restored dune soils, I conducted two greenhouse experiments: (1) a bulk sand experiment to measure broad effects on the growth of native plant species, and (2) a microbial study to specifically test for the effects of soil microbes in the different treatments on the growth of native plants (Table 1). Although a greenhouse experiment lacks many aspects of the natural dune system such as ocean spray, wind, and sand movement, it allows for a replicated study of relative differences between treatments.

For both studies, I collected seeds from *L. carnosa* in June to July 2012 from the Abbott's Lagoon dunes, and *M. undulata* in late July to early August within one mile south of Abbott's Lagoon. I stored the seeds in paper envelopes in a sealed plastic box at PRNS headquarters until they were planted and grown in the Oxford Tract greenhouse (UC Berkeley) in the Fall of 2012. I collected sand from the Abbott's Lagoon dunes and extending south within one mile of the lagoon (Fig. 1) on September 30 and October 5, 2012, and stored it in a refrigerated room at the greenhouse until planted, to inhibit microbial activity and minimize changes from field conditions.

In order to simulate natural growing conditions as much as possible, I used a strict care regimen. I lightly watered the plants daily with deionized water to keep the top layer of sand moist. Plants were grown in 2.5" diameter by 10" deep containers, with sterile cotton balls placed in the bottom to hold the sand in. I mixed vermiculite in the bottom 6 inches of the sand to maintain some soil moisture. I arranged containers in trays that served as blocks, which I rotated and randomized periodically. The greenhouse was temperature and light regulated. Initially I placed the cones in a room with daylight for 8 hours per day to mimic more closely the shorter winter hours; if light levels dropped below 1000 mW, overhead lights turned on.

Due to low germination in the original setup, I restarted the experiment in the same sand by transplanting seedlings grown in sterile sand. All transplanting was done with care to preserve as many roots as possible. During this second trial the cones were placed in a different room with 16 hours of daylight to encourage more growth; the room had the same light controls as the previous. These plants yielded sufficient replicates to harvest individuals.

Table 1. Greenhouse study treatments and parameters. Native treatment is defined as soil taken from the native dune area south of Abbott's Lagoon, in dune mat habitat. Flipped restored treatment is from the Abbott's Lagoon Restoration Project. The *C. edulis* without duff treatment is from a patch of *C. edulis* that then had the roots and duff scraped away. The *C. edulis* with duff treatment is the same as the previous, but with duff collected and replaced in the cone when in the greenhouse. The microbe study has similar treatments using only a small inoculum; the control is a mix of the three field soils sterilized in the autoclave.

	Soil Treatments	Blocks	Seeds/cone	Species
Nutrient/Bulk study	(1) Native (2) Flipped restored (3) *C. edulis* without duff (4) *C. edulis* with duff	7 trays	5	*L. carnosa and M. undulata*
Microbe/Inoculum Study	(1) Control (2) Native (3) Flipped restored (4) *C. edulis*	9 trays	4 sterilized	*L. carnosa* and *M. undulata*

Bulk sand study

To measure soil effects of *C. edulis* removal and flipped sand on the growth of native plants, including changes in soil nutrients and microbial interactions, I conducted a bulk sand study. I collected soils with four treatments: (1) Native control: a control of native dune soil; (2) Flipped restored: from the flipped restored area south of Abbott's Lagoon; (3) iceplant with duff: from under *C. edulis*, take topsoils (top 15 cm), remove *C. edulis*, scrape off the organic layer to preserve it separately and replace it in the pots in the greenhouse; and (4) iceplant without duff: from under *C. edulis*, take topsoils (top 15 cm) and remove *C. edulis* and the organic layer (Table

1). In each cone I planted five unsterilized seeds under a small layer of sand. Two weeks later I planted 22 *L. carnosa* (the maximum allowed under the FWS permit) and 60 *M. undulata* seeds in a flat of sterile sand for transplanting if needed. Three weeks after initial planting I transplanted seedlings from containers with extra seedlings to containers that had no germination within the same treatment to have more complete replicates.

I measured total germination, seedling height (mm) for *M. undulata* and rosette diameter (mm) for *L. carnosa*, and above ground biomass (mg). Above ground biomass was measured as the plant matter above the level of the sand harvested nine weeks after starting the second trial.

To determine the effects of the treatments in the study I looked at germination and above ground biomass. I measured total germination using a general linear model with a logistic regression with species and soil type as the predictor variables. I log transformed the biomass data and used a two-way ANOVA with block as random term, initial height/width as covariate, species with two levels, and soil type with four levels as the explanatory variables.

Microbe study

To test whether differences in growth resulted from microbial interactions isolated from changes in nutrients in the sand, I conducted a soil inoculum study. I sterilized store-bought sand in an autoclave at 250º F for 4 hours and inoculated it with 2 tablespoons of sand from the field in four treatments: (1) iceplant: from *C. edulis* patches, (2) flipped: from the flipped restoration site at Abbott's Lagoon, (3) native: from native dunes at Abbott's Lagoon, and (4) control: a mixture of the other three treatments that I then sterilized in the autoclave for .5 hours at 250º F (Table 1). I collected sand samples using gloves for each soil type into one Ziploc bag per treatment, sterilizing all tools with ethanol between treatments. I collected and cut up bits of fine roots, if present, to add to the inoculum of 2 tablespoons at the top of the container. For each treatment I planted *L. carnosa* (4 seeds per container) and *M. undulata* (4 seeds per container) separately. I sterilized the seeds in the lab using a 10% bleach solution in deionized water for 5 minutes.

Two weeks after initial planting I planted 25 sterilized *L. carnosa* and 60 sterilized *M. undulata* seeds in a flat of sterile sand for transplanting if needed. Three weeks after initial planting I transplanted seedlings from containers with extra seedlings to containers that had no germination within the same treatment to have more complete replicates. I conducted a second trial with transplants and harvested above and below ground biomass 9 weeks after planting.

I measured plants in each treatment for total germination, growth (as measured by height for *M. undulata* and rosette diameter for *L. carnosa*), and above and below ground biomass. Total germination was analyzed using a general linear model with a logistic regression with species and soil type as the predictor variables. For above ground biomass, I log transformed the data and used a two-way ANOVA with block as random term, initial height/width as covariate, species with two levels, and soil type with four levels as the explanatory variable.

RESULTS

Soil characteristics

I found significant differences in soil nutrients between native soil and *C. edulis* soil and varying degrees of edge effects (Table 2). Specifically, I found significantly lower pH, sodium, and magnesium levels in *C. edulis* soil than in native, and significantly higher potassium and percent organic matter in the *C. edulis* than in native soil ($p < 0.5$) (Table 2). Gradient edge effects as seen with pairwise Tukey HSD tests were statistically significant in sodium between all three soil types, with sodium decreasing significantly with progressive invasion of *C. edulis* ($p < 0.05$ for each pairwise test) (Figure 2). Pairwise tests (Tukey HSD) of pH show significantly lowered pH in *C. edulis* soils from native ($p = 0.012$, $t = 3.1$) but no significant edge effects (Table 2). Although not all edge measurements showed statistical difference, many measurement averages of edge soils do lie between native and *C. edulis*.

Table 2. Summary of soil variable results. Under each soil type is the mean and standard error along with a letter corresponding to Tukey HSD results. Soil types within each measurement with the same letter are not significantly different; those with different letters are significantly different ($p < 0.5$). For ANOVA results see Appendix A.

Soil Variable	**Native**	**Edge**	**C. edulis**
pH	5.24 ± 0.09 a	4.98 ± 0.13 ab	4.67 ± 0.17 b
Cation Exchange Capacity	1.62 ± 0.1	1.83 ± 0.2	1.81 ± 0.2
Calcium	81.75 ± 3.7	79.08 ± 5.7	83.00 ± 8.9
Hydrogen	0.58 ± 0.1	0.83 ± 0.1	0.98 ± 0.2

Magnesium	54.08 ± 1.3 a	49.92 ± 2.1 ab	37.50 ± 4.3 b
Phosphorus (weak Bray)	9.67 ± 1.3	12.25 ± 2.1	13.58 ± 2.3
Potassium	19.67 ± 1.6 a	40.25 ± 6.8 b	32.17 ± 3.0 b
Sodium	35.92 ± 4.8 a	18.92 ± 3.15 b	6.67 ± 1.1 c
Sulfate	3.42 ± 0.4	3.33 ± 0.6	2.58 ± 0.3
% Organic Matter	0.16 ± 0.03 a	0.23 ± 0.04 ab	0.30 ± 0.05 b
Organic Matter (ENR)	33.42 ± 0.6	34.58 ± 0.8	35.92 ± 1.1

Greenhouse: bulk sand experiment

I found both species had significantly higher germination in native and flipped soils than in either of the *C. edulis* treatments ($p < 0.001$) (Table 3). Of the two *C. edulis* treatments, two seeds of *L. carnosa* germinated in one cone of the duff treatment. In the logistic regression both species had significantly higher germination in the native treatment than the flipped treatment ($p = 0.04$).

Table 3. Germination of each species per soil type.

Species	Native	Flipped	Duff	No duff
L. carnosa	57.5%	30%	0%	6%
M. undulata	72.5%	57.5%	0%	0%

Above ground biomass was significantly affected by all factors in the test (Table 4). Shoot biomass of *L. carnosa* significantly differed ($p < 0.001$) between soil types however there were no significant differences for *M. undulata* (Table 5). A significant species by soil interaction shows that the two species responded differently to soil types ($p = 0.031$). Using least square means, which account for initial width of *L. carnosa* as well as unequal replication, plants in the native soil grew 116% greater biomass than in flipped soil and 132% higher in native than *C. edulis* with duff; plants in the treatment without duff had 142% greater biomass than with duff present. *Carpobrotus edulis* treatment with duff showed no significant difference from native soil, however *L. carnosa* had 43% greater above ground biomass in the *C. edulis* without duff treatment than with duff treatment ($p = 0.007$).

Table 4. Fixed effect test with log transformed shoot biomass of *L. carnosa* and *M. undulata* as the response variable, with block as random term, initial height/width as covariate, species with two levels, and soil type with four levels. For the number of parameters associated with an effect, continuous effects have one parameter and ordinal effects have one less parameter than the number of level. The F statistic is the ratio of the mean square of the effect divided by the mean square for error.

Source of variation	Number of parameters	*df*	df_{den}	F Ratio	*P* value
Initial height/width	1	1	25.14	32.9228	<.0001***
Species	1	1	22.88	6.1714	0.0207*
Soil type	3	3	22.48	5.4696	0.0056**
Species:Soil type	3	3	22.86	3.5337	0.0308*

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05

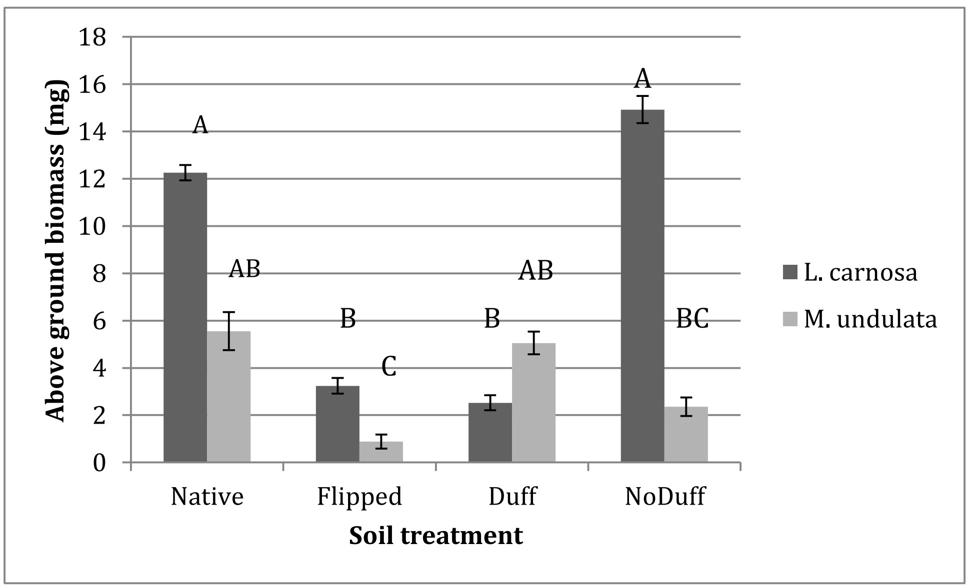

Figure 2. Shoot biomass (mg) least square mean and standard error for each species in each soil treatment for the bulk sand study. LSM was used to account for initial height/width and unequal replicates. Significance between treatments as calculated by the student’s t-test is noted by letters that are not shared ($p < 0.05$); letters shared between treatments signify that they are not significantly different.

Greenhouse microbe experiment

In the initial study both species had significantly higher germination in the control, native, and flipped soils than in the *C. edulis* inoculation, in which one *L. carnosa* seed germinated ($p < 0.001$). Plants had significantly higher germination in the native and flipped treatments than in the control ($p < 0.001$ for native and $p = 0.02$ for flipped inoculum). Differences between native and flipped treatments were nonsignificant. There was no species by soil interaction.

Table 5. Germination of microbe experiment.

Species	Control	Native	Flipped	*C. edulis*
L. carnosa	44.4%	55.6%	33.3%	2.8%
M. undulata	16.7%	66.7%	63.9%	0%

I found that plants grown in flipped soil produced significantly less biomass than in any of the other treatments ($p < 0.05$ for each), although I found no significant differences between other soil types (Fig. 2). I did not observe a species by soil interaction effect. Plants in the native soil grew 75% more in the native treatment than the flipped treatment.

Table 6. Fixed effect test with log transformed shoot biomass of *L. carnosa* and *M. undulata* as the response variable, with block as random term, initial height/width as covariate, species with two levels, and soil type with four levels. For the number of parameters associated with an effect, continuous effects have one parameter and ordinal effects have one less parameter than the number of level. The F statistic is the ratio of the mean square of the effect divided by the mean square for error.

Source of variation	Number of parameters	*df*	df_{den}	F Ratio	*P* value
Initial height/weight	1	1	50.81	14.9881	0.0003***
Species	1	1	50.38	41.1094	< 0.0001***
Soil type	3	3	49.75	6.5100	0.0008***
Species:Soil type	3	3	49.43	2.2569	0.0934

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

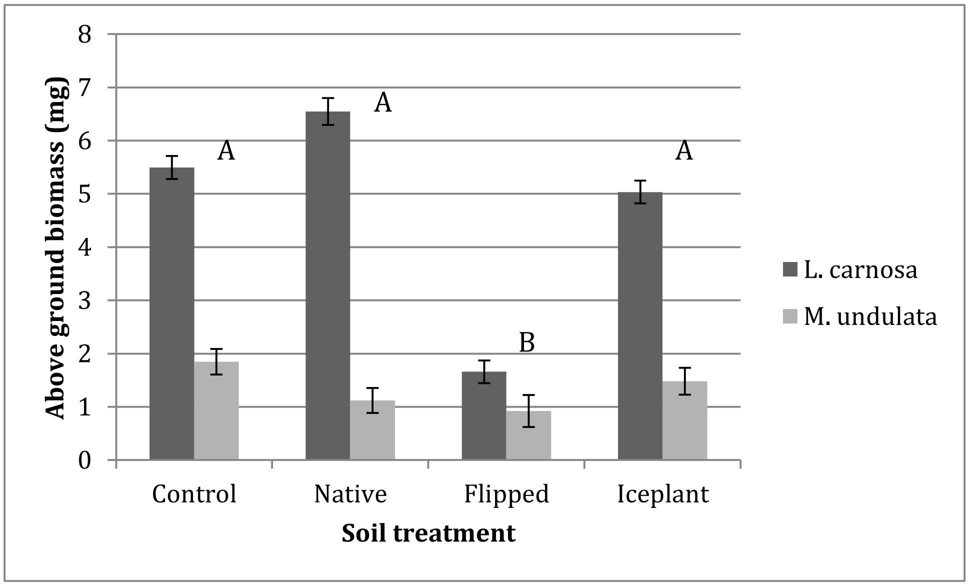

Figure 3. Shoot biomass (mg) least square mean and standard error for each species in each soil treatment of the microbe study. No significant species:soil interaction was found. Levels not connected with the same letter are significantly different ($p < 0.05$).

DISCUSSION

Restoration methods affect the success of germination and growth of native plants, and appear to have effects on multiple levels due to plant-soil feedbacks. The soil characteristics of *C. edulis* and native soils differ in key ways, confirming that it changes soil nutrients; it also inhibits the germination of native plants and greatly reduces their growth. The flipped soil also showed reduced germination and growth in both of the greenhouse studies. The projects show that the flipped soil may have many consequences for the restoration of native plants, as do removal of *C. edulis.* All of the restoration methods I studied indicate that time and more rehabilitation will be required in order for native plants to reestablish.

Soil characteristics

Many studies in different ecosystems have shown that exotic species can change soil conditions, in the form of alterations to the physical properties of the soil or the soil microbial community, connected with the changes in inputs to the soil from the exotic plant (D'Antonio and Haubensak 1998, Ehrenfeld 2003). *Carpobrotus edulis* has been seen to lower pH and increase organic matter, which is often understood as a mechanism to enhance its own growth (Conser and Connor 2009, de la Peña et al. 2010, Santoro et al. 2011). Sodium, magnesium, and potassium all differ significantly between soil type; they are all cations found in sea spray. In other ecosystems sodium has been measured to on average increase under *C. edulis*, while I measured a significant decrease under *C. edulis* with a strong gradient; this should be studied more between different types of dune systems (Molinari et al. 2007). While cation exchange capacity showed no difference between soil types, the individual cation concentrations did show some significant differences.

Measurements generally showed a gradient with edge effects between the native and *C. edulis* results. When there was a significant difference between the native and *C. edulis* soils for pH, magnesium, and organic matter, the edge measurement was not significantly different from native or *C. edulis* soil. The trend, however, suggests that a gradient exists, as the mean of the edge sample lies between the native and *C. edulis* measurements. For sodium, all three measurements are significantly different and clearly show a progressive gradient.

Bulk sand treatments on the growth of native plants

Germination in the native soil was significantly higher than the flipped soil, while no seedlings germinated in either of the *C. edulis* treatments. The inhibition of germination in the *C. edulis* soil may be due to the decrease in pH as seen in the soil sampling (Novoa et al. 2012, Unpublished C. D'Antonio, B. Dallabona, and V. Vincent).

When adjusted for initial height/width and uneven replication, *L. carnosa* showed significantly higher above ground biomass in native soil and *C. edulis* without duff than in the flipped and with duff measurements. This points to the possibility that the breakdown of litter from *C. edulis* causes changes in the soil composition that inhibit the growth of native species (Molinari et al. 2007, Novoa et al. 2012). Carpobrotus *edulis* modifies its habitat with plant-soil feedbacks as a method of negative feedback, by inhibiting other plants; in the bulk sand study, this may be seen as coming from microbial effects, soil chemistry, or allelopathic chemicals (Ehrenfeld 2003, Conser and Connor 2008, Novoa et al. 2012).

Inoculum microbial effects on the growth of native plants

Germination was significantly higher in native and flipped treatments than in the control, which shows that microbes in the soil may be beneficial for germination of native plants. The *C. edulis* treatment yielded only one seed germinated, leading to the conclusion that *C. edulis* has negative microbial interactions or possibly allelopathic interactions—because the seeds are only at the surface for germination, effect cannot be attributed to microbes with the exception of comparisons to the sterile control.

Soil microbial communities often play an important role in the growth of plants (Ehrenfeld 2003). I found that the sterile control, native, and *C. edulis* treatments did not differ significantly in above ground biomass, while plants in the flipped soil grew significantly less than all of them. These results show that the flipped soil has parasitic microbes that reduced the growth of plants. With the small amount of field sand in each inoculum, soil nutrients and allelopathic compounds would be in small enough amounts to not cause a significant change.

Native species growth after restoration

Soil treatments of *C. edulis* and flipped soil had varying degrees of effects on the germination and growth of *L. carnosa* and *M. undulata* in the two studies. The *C. edulis* without duff treatment showed significantly higher above ground growth than *C. edulis* with duff, and a similar level to growth in the native treatment. In the microbe study, *C. edulis* did not differ significantly from the native or control treatments, which shows that microbes likely do not play a significant role in the decreased growth of native plants in *C. edulis* soil.

In both studies, plants grew significantly more in native soil than flipped soil. For the bulk sand study, *L. carnosa* grew 116% more in native soil and *M. undulata* grew 145% more in native soil; in the microbe study, the two plants together grew 75% more in native soil than flipped. There was no statistical species by soil type interaction, but *L. carnosa* had 119% greater above ground biomass and *M. undulata* had 19% greater above ground biomass in native than flipped soil. The similarities in these effects show that microbes play a large part in the lowered biomass of plants in flipped soil. Soil chemistry and allelopathic chemicals would not have a significant effect from the small inoculum; in the bulk sand study, a significant portion of the change likely came from microbial effects in the soil.

In the *C. edulis* treatments, microbial effects may play a significant role in the growth of native plants, but with more variable results. Comparing the treatment of native soil to that with no duff due to the possibility of microclimate effects in the duff treatment, I found a 39% larger difference in the bulk sand study compared to the microbe study for *L. carnosa*. With *M. undulata*, however, the treatment with no duff actually had 7% greater average biomass than in the native soil. In the microbe study, biomass of native plants had 33% greater growth in the native soil than the *C. edulis* treatment. The two plants show different reactions to the *C. edulis* treatments, and vary in relation to the effects of microbes. This may indicate that the microbial effects negatively affect both species in *C. edulis* treatments, though there are other factors such as soil chemistry and allelopathic chemicals that may also contribute to the inhibition of the growth of native plants (Novoa et al. 2012).

Limitations and future directions

Working with rare species limited my access to seed material. In the greenhouse studies, I had low germination and in future research would try seed preparation methods to have more germination and better replication (Maun 2009). As *L. carnosa* is an endangered species, I had a limited numbers of seeds available to me under the USFWS permit.

In future research, the Abbotts Lagoon Coastal Dune Restoration Project should be monitored for seedling emergence and more research could be done on ways to make the area more hospitable for native plant germination and seedling survival. Studies should also be conducted on the response of *C. edulis* to the different treatment areas, to see if it changes the area to help itself grow and to test for the potential of invasion of the flipped area by *C. edulis*. Field studies should also be conducted to see how the plant-soil feedbacks observed interact with other elements such as competition and wind. Allelopathy could be tested in the future by neutralizing any possible chemicals with activated carbon, which would provide a new layer of knowledge to the interaction of *C. edulis* and plant-soil feedbacks.

Management applications

Invasion of *C. edulis* leads to changes in the ecosystem that affect the growth of native species after its removal. Only one native seed germinated in any of the *C. edulis* treatments; while transplanted seedlings showed some growth, if they are not able to get a hold, there will be little hope for their recolonization. The growth of the plants was higher in the treatment without duff than with duff still in place, though germination for both was low; the microbe study also shows significant decrease in growth, which points to microbial interactions of the duff as a possible cause of decreased growth. There are multiple ways this could be applied to management. With the roots and duff still in place, the sand does not regain the same mobility of native sand dunes; if the roots and duff were all removed, it is possible that the turnover of the sand would happen more rapidly and allow native plants to recolonize more quickly. As a different method of soil rehabilitation, inoculation is a common practice in grasslands and other ecosystems to start a more hospitable microbial community. If *C. edulis* roots were left in place, it may be possible that the sand could be inoculated with native microbes. If the roots and duff were removed, the high mobility and rapid turnover of the sand could make this method ineffective. The spraying of *C. edulis* with herbicide would likely inhibit the germination and growth of native species for significantly longer than pulling it; the decomposing matter has been seen to cause lasting effects on the soil and the high amount of duff would be a difficult environment for small native plants to grow through.

With the flipping restoration method at the Abbotts Dunes Project, the plants need to survive a parasitic microbial community in addition to the high winds and rapid turnover of sand. With only one species, *Lupinus tidestromii*, currently colonizing the area in the first year after the project, there can be hope that in the coming years more plants will colonize the area and return a native microbial community while stabilizing the sand. The prospect of inoculation would not be feasible with the rapid turnover of sand. It may be important to transplant perennial plants from the native dune that could help to stabilize the mobile sand and bring any mutualistic microbes with it. The removal of *Ammophila arenaria* by flipping may be a more effective restoration than spraying with herbicide (as the alternative), as it removes the high biomass of *A. arenaria* and opens up the sand that is naturally mobile.

This project shows that there are consequences of restoration that affect the establishment and growth of native plants. Restoration is important for making an area available for the growth of native plants, but more than just removal of invasives, there are factors such as microbial communities that are often seen as less important that the above-ground plants. This project shows that the two cannot be separated, and the dune microbial community plays an important role in the growth of native plants.

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APPENDIX A: Soil Nutrient Analyses

Table A1. Summary of ANOVA results for all tested soil nutrients.

Soil variable	df	F	P
pH	2, 33	4.73	0.016 *
Cation Exchange Capacity	2, 33	0.57	0.569
Calcium	2, 33	0.10	0.909
Hydrogen	2, 33	2.33	0.113
Magnesium	2, 33	6.84	0.003 **
Phosphorus (weak Bray)	2, 33	1.56	0.226
Potassium	2, 33	8.91	<0.001 ***
Sodium	2, 33	21.16	<0.001 ***
Sulfate	2, 33	1.12	0.338
% Organic Matter	2, 33	4.25	0.023 *
Organic Matter (ENR)	2, 33	2.58	0.091

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05